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MICROBIAL OIL MIXTURES AND USES THEREOF

This invention relates to blends or mixtures of polyunsaturated fatty acid-containing microbial oils and to uses thereof. In a specific preferred embodiment, this invention concerns the use of such oils as an additive or supplement for human diets, for example, as an additive to infant formula.

It long has been known that long chain polyunsaturated fatty acids (PUFAs) are essential to the human diet, particularly during periods of rapid tissue growth. Sanders et al, Am. J. Clin. Nutr., 31:805-813 (1978). Certain of these long chain acids, such as arachidonic acid (ARA), cannot be synthesized de novo in humans. Only by metabolizing linoleic acid (LOA), which is connected to gamma linolenic acid (GLA), and then to ARA can the human body produce ARA. LOA, in turn, is an essential acid which can only be obtained from dietary sources. Additionally, the presence of eicosapentaenoic acid (EPA) in the diet inhibits the metabolic conversion of LOA to ARA. Carlson, et al., INFORM, 1:306 (1990). ARA and docosahexaneoic acid (DHA) are critical elements of muscle, organ and vascular tissues.

Infancy is the most significant period of rapid growth in a human's life. An infant can increase its body weight by three times or more during its first year of life. Accordingly, it is critical that the infant receive adequate amounts of PUFAs to insure

proper structural and organ development. Human breast milk contains high levels of PUFAs in which the ratio of ARA to EPA is typically about 20:1. However, many women choose not to breast feed their infants for either part or all of the first year of the infant's life.

As recognized by Clandinin et al., U.S. Patent 4,670,285, incorporated herein by reference, available infant formulas are deficient in long chain (C_{20} and C_{22}) PUFAs. Clandinin et al. disclose an infant formula prepared from a blend of vegetable oil and egg yolk lipid and/or fish oil which can provide a total fat composition comparable to that of human breast milk. A preferable composition comprises from 75 to 95 parts by weight egg yolk and 5 to 25 parts vegetable oil. This composition is the entire lipid content of the infant formula and it is not economical to prepare. Additionally, the infant formula disclosed by Clandinin et al. results in an EPA level which is 16 times higher than the level of EPA in human breast milk and an ARA level which is only one quarter that of breast milk.

DE 3603000A1 (Milupa) discloses a computer profile of a highly polyunsaturated acid fat mixture and discusses the use of such a mixture to produce infant formulas. Sources of the fatty acids are listed as certain types of macroalgae (i.e. seaweed), fish oil, organ fats from beef and pork, and highly refined egg yolk oil. In addition to DHA from fish oil, a potential source of DHA and ARA is said to be macroalgae, but only of the seaweed types. There is no suggestion to use microbes of any type, much less microbial oil.

Methods of producing microbial oils are disclosed in the following references, each of which is

incorporated herein by reference. Co-pending U.S. Patent Application 07/496,572, filed March 21, 1990, now Pat. No. 5,244,921 discloses the production of eicosapentaneoic acid-containing single cell oils (EPASCO). Co-pending U.S. Patent Application 07/479,135, filed February 13, 1990, discloses the production of docosahexaneoic acid-containing single cell oil (DHASCO). Co-pending U.S. Patent Application 07/645,454, now abandoned ~~[serial number to be inserted when assigned]~~, filed concurrently with the present application and assigned to the same assignee, relates to the production of arachidonic acid-containing single cell oil (ARASCO). EP322,227 also discloses a microbial oil production system. None of these references teach the use of blends containing unmodified microbial oils as a dietary supplement, or the use of a blend of microbial oils as an additive to existing infant formula to provide that formula with a long chain PUFA composition similar to breast milk.

Accordingly, it is an object of the present invention to provide a PUFA-enriched additive, the composition of which when added to commercial infant formula will provide desired long chain PUFAs in amounts comparable to the amounts of those PUFAs found in human breast milk.

It is an additional object of the present invention to provide an economical method of producing the above-described composition.

These, and other, objects are satisfied by the present invention as described herein.

Summary of the Invention

This invention relates to the use of microbial oils which contain long chain polyunsaturated fatty acids. Additionally, in various embodiments, fish oil

and/or vegetable oils can be blended with such microbial oils to form desired compositions. The compositions can be used as dietary supplements, particularly as additives for infant formula, as well as for pharmaceutical and cosmetic applications.

The invention also relates to economically viable processes for altering the long chain polyunsaturated fatty acid composition of infant formula and/or baby food. Preferably, the altered composition resembles that of human breast milk.

Detailed Description of the Preferred Embodiment of the Invention

Broadly stated, the present invention concerns blends, or mixtures, containing unmodified microbial oils. As used herein, "unmodified" means not chemically or covalently altered. It will be understood that throughout this specification references to "microbial oil" or "oil" mean, unless otherwise specifically stated, unmodified oil. "Microbial oils" or "single cell oils" are those oils naturally produced by microorganisms during their lifespan. Such oils can be high in long chain PUFAs. The applicant has discovered that certain of these oils, when blended with other microbial oils, fish oils, vegetable oils, or any combination thereof, can produce a composition useful for dietary, pharmaceutical or cosmetic purposes.

Various microbial oils, for example, can be obtained by, for example, the processes disclosed in above-referenced U.S. Patent Applications 07/496,572, now Pat. No. 5,244,921, 07/479,135, EP322,227 (Yamada et al., Suntory) or U.S. Patent Application ~~[Serial number to be provided when issued by USPTO]~~ 07/645,545 454, now abandoned, (the latter having been filed

concurrently with the present application and assigned to the same assignee). The disclosure of each of these references is specifically incorporated by reference herein.

It is to be understood that the present invention encompasses the use of a single-microbial oil containing at least two desirable PUFAs, such as ARA and DHA. The oils specifically disclosed and utilized herein, however, each contain a single desirable PUFA.

Any non-toxic, PUFA-containing microbial oil can be used in the present invention. The most preferred microbial oils are those rich in an omega-3 or omega-6 PUFA, especially DHA, GLA or ARA. These PUFAs typically are missing from, or are inadequately provided in, dietary supplements such as infant formulas or baby food. "Infant formula" as used herein means an enteral nutritional product which can be substituted for human breast milk in feeding infants and typically is composed of a desired percentage of fat mixed with desired percentages of carbohydrates and proteins in an aqueous solution. Frequently micronutrients, such as trace metals and vitamins or other desired additives are present. An exemplary formula is disclosed by Clandinin et al., U.S. Patent No. 4,670,285, the disclosure of which is incorporated herein by reference.

In the present invention, types of oils from different microbes can be mixed together to obtain a desired composition. Alternatively, or additionally, PUFA-containing microbial oil can be blended with fish oil, vegetable oil or a mixture of both to obtain a desired composition.

An objective in mixing the oils is to obtain an additive which will provide an infant formula with a

desired omega-3 and omega-6 PUFA composition similar to that found in breast milk. While the proportion of the desired fatty acids in a microbial oil can vary, this proportion can easily be determined and the amount of oil adjusted to provide the desired amount of PUFA. Similarly, the percentage of desired PUFA in fish oil or vegetable oils can easily be determined and the amount of the oil to be added can be adjusted as necessary to achieve the desired results.

"Fish oils" are those oils obtained from fish. Such oils typically contain DHA in amounts ranging from 3% to about 20%. Typically, however, fish oils also contain EPA which depresses the production of ARA in the body. The addition of a microbial oil containing high levels of ARA to fish oil-containing compositions substantially overcomes that problem.

"Vegetable oil" includes all those oils from plants which contain ~~long-chain~~ PUFAs. Typically, vegetable oils do not contain long chain PUFAs (PUFAs at least 20 carbons long), which is why animal organ oils are usually characterized as the source of PUFAs. Thus, vegetarians, especially vegetarian mothers, can have a diet containing inadequate amounts of PUFAs. Vegetable oils known to contain PUFAs may contain GLA. GLA is a C18:3 omega-6 PUFA. Such oils include black currant seed oil, borage oil and primrose oil. While GLA is the metabolic precursor to ARA, the process of conversion is very slow, requiring the participation of the enzyme Δ6-desaturase. This enzyme is present in humans in very low levels. Burre, et al., Lipids, 25:354-356 (1990). Thus, it would be preferable to provide the body with ARA rather than its precursor, GLA.

Methods for isolating vegetable oils are known to those of skill in the art and do not comprise a part of the present invention. Additionally, certain fungi produce PUFA-containing oils. For example, *Mucor* species produce a GLA-containing oil.

DHASCO, defined herein as docosahexaneoic acid-containing single cell oil, can be obtained, for example, from *Crypthecodinium cohnii* as disclosed in above-referenced U.S. application 07/479,135. DHA is a C22:6 omega-3 long chain PUFA.

EPASCO, defined herein as eicosapentaneoic acid-containing single cell oil, can be obtained, for example, from *Nitzschia alba* as disclosed in above-referenced U.S. application 07/496,572, now Pat. No. 5,244,921. EPA is a C20:5 omega-3 long chain PUFA.

ARASCO, defined herein as arachidonic acid-containing single cell oil, can be obtained from species such as *Pythium insidiosum*, or *Mortierella alpina*, as described in U.S. application 07/645,454, now abandoned, filed concurrently herewith. ARA is a C20:4 omega-6 long chain PUFA.

Another aspect of the invention discloses a process for supplementing or altering the composition of commercially available infant formula so as to provide them with a PUFA composition more nearly like that typically contained in human breast milk. "Typical" as used herein refers to the average amounts of PUFAs measured. One of the advantages of the present invention is that, if desired, a nursing mother choosing to switch to formula can have her breast milk analyzed for PUFA content. Then, an additive for a commercially available formula which will supply comparable amounts of PUFAs can be specifically designed. Long chain PUFA-containing microbial oils

from at least two microorganisms can be obtained and blended together to provide the desired composition. The blend then can be added to an infant formula. Preferably, an amount of the blend effective to provide an amount of the desired PUFAs substantially similar to that found in human breast milk will be provided.

Typically, human breast milk contain from about 0.5 to 0.6% of its fatty acid content as ARA, from about 0.15 to about 0.36% of its fatty acid content as DHA and from about 0.03 to about 0.13% of its fatty acid content as EPA. Thus, a preferred ratio of ARA:DHA:EPA is from about 5:1:1 to about 20:10:1 respectively. Amounts of oils providing approximately these ratios of PUFAs can be determined without undue experimentation by those of skill in the art.

In a preferred embodiment, the microbial oils include ARASCO and DHASCO and EPASCO or any combination thereof. It is also preferred to use oil from microbes of the genera *Mortierella*, *Pythium*, *Crypthecodinium*, and *Nitzschia* or any combination thereof. Particularly preferred species from these genera are *M. alpina*, *P. insidiosum*, *C. cohnii* and *N. alba*. This preferred embodiment would provide an acceptable alternative for vegetarians, including breast-feeding or pregnant vegetarian women.

If desired, fish oil can be blended, or mixed, with any combination of, or individual, microbial oil to produce a composition which, when subsequently added to infant formula will alter the PUFA content thereof in a desirable manner. Such a composition would not be suitable for a strict vegetarian intake. A preferred fish oil is specially processed Menhaden Oil (produced by Zapata Hayne, Inc.) which typically contains about 9% DHA. Of course, other fish oils also can be used.

When DHASCO is to be blended with ARASCO, and no other PUFA-containing oils are to be utilized, it is desirable to blend sufficient amounts of the oils to provide from about 1 to about 5 parts DHA with from about 2 to about 12 parts ARA. A most preferred ratio of DHA to ARA is 1:3 respectively.

As another example, Menhaden fish oil, as noted above, typically contains about 9% by weight DHA. ARASCO typically contains about 20 - 40% by weight ARA. DHASCO typically contains about 25 - 40% by weight DHA. It has been found that a blend of 1 part Menhaden oil containing about 9% by weight DHA with 10 parts ARASCO containing about 33% by weight ARA and 3 parts DHASCO containing about 35% by weight DHA, when added to infant formula, causes the infant formula to closely approximate the ARA and DHA content of human breast milk. Other ratios can be readily calculated.

In another embodiment of the present invention is disclosed a process for making a supplement for infant formula or baby food which entails blending a DHA-containing oil with a GLA-containing oil. It is to be understood that, in general, any combination of GLA- EPA- ARA- or DHA-containing oils, with or without fish oil, can be used. The source of the GLA can be a vegetable oil, such as primrose, black currant or borage oil, or a microbial oil such as the oil from *Mucor javonicus* or *Mortierella isabellina*, for example. Table 1 sets forth the GLA composition of such oils. In a preferred aspect of this embodiment, about 1 part of Menhaden oil containing about 9% DHA, about 4 parts of GLA-containing oil containing about 18% GLA from black currant seed, and about 1 part of DHASCO containing about 33% DHA are blended together. Other ratios can be selected as desired.

Table 1. Fatty acids of commercially available oils containing GLA (from Lawson and Hughes, 1988 and Suzuki, 1989)

Fatty acyl group	Relative % of total acyl groups in oil from: Mucor javanicus*	Mortierella isabellina**	Evening Primrose	Black-currant	Borage
14:0	1.0	0.7	-	-	-
14:1	0.1	-	-	-	-
16:0	18.6	27.2	5.9	6.9	10.7
16:1	1.0	0.9	-	-	-
18:0	7.1	5.7	1.8	1.3	3.0
18:1	39.9	43.9	7.5	10.8	15.4
18:2	8.9	12.0	74.8	46.7	38.1
γ-18:3(ω6)	17.9	8.3	9.3	15.9	24.8
α-18:3(ω3)	-	-	-	13.0	-
18:4(ω3)	-	-	-	2.9	-
20:0	-	0.6	-	-	-
20:1(ω9)	-	-	-	-	4.0
22.0	-	0.1	-	-	-
22:1(ω9)	-	0.2	-	-	2.2
24:0	0.6	-	-	-	-

* Produced by J. & E. Sturge Ltd., Selby, N. Yorks., U.K.
** Produced by Idemitzu Petro Chemical Co. Ltd., Tokyo, Japan.

Lawson - Lipids 23:313-317 (1988)
Suzuki - In Biotechnology for the Fats and Oils Industry p.110-116. Amer Oil Chem. Soc. Press (1989).

A composition including a blend of any combination of the above-described microbial oils with or without either, or both, fish oil and vegetable oil is another aspect of the present invention. While the composition includes any ratios of the oils, the ratios previously described are preferred.

In another preferred embodiment, the composition serves as a nutritional supplement. Typically, such supplements are encapsulated, such as in gelatin capsules. Such capsules provide an easy form of administration to persons having a need for supplementation, such as pregnant or nursing women. However, parenteral administration is a viable option and in one embodiment the composition comprises the fat component of a total parenteral nutritional formula. Such formulas are known and commercially available.

As will be understood, the composition of the present invention is particularly useful as a dietary supplement for pregnant or nursing women. Vegetarian women, in particular, may require increased amounts of DHA and ARA, yet have been precluded from obtaining such in the past because the only available sources were animal.

The invention having been previously described in general, reference is now had to the following non-limiting examples for illustrative purposes only.

Examples

Example 1. Preparation of Oil Mix #1 and addition to infant formula.

The first mixture represents a totally vegetarian source of an arachidonic and docosahexaenoic acid supplement. This supplement would be considered acceptable to persons restricted to a vegetarian diet. Sanders et al. (Amer. J. Clin. Nutr. 31:~~705~~ 805; 1978) have

reported that the DHA levels in the breast milk of vegetarian mothers are depressed. Enteral supplementation of a blend of DHA single cell oil and ARA single cell oil will elevate the serum and, hence, breast milk levels of DHA to that of omnivorous mothers. This blend is prepared by mixing one part DHASCO containing about 35% DHA (obtained from *Crypthecodinium cohnii* as described in ~~patent application #07/479-135~~ Example 3 and deposited with the American Type Culture Collection in Rockville, Maryland (ATCC) having accession number 40750) with three parts ARASCO containing about 33% ARA (obtained from *Pythium insidiosum* as described in patent application # Example 2 and on deposit with the ATCC, having accession number 28251). The resulting mixture, or blend, has the fatty acid composition shown in Table 2. The blend is mixed in a ratio of one part blend to forty parts of the oils regularly in infant formula, typically about 2.8 - 3.0 grams per 100 ml of formula. At a normal fat content of 30g fat per liter of Similac® infant formula, this corresponds to the addition of 750 mg per liter of prepared formula. This supplement provides ARA and DHA levels equivalent to human breast milk.

Table 2. Composition of a blend of DHA oil and ARA oil in proportions of 1:3 by weight.

Fatty Acid	oil mix #1	Infant formula	formula + mix #1	breast milk
8:0 + 10:0	0.00	41.8	40.78	1.74
12:0 + 14:0	13.63	20.7	20.53	14.95
16:0	17.05	6.8	7.05	19.82
16:1	7.88	0.2	0.39	3.20
18:0	0.00	2.3	2.24	5.91
18:1	7.48	10.0	9.94	34.82
18:2 n6	7.20	17.4	17.15	16.00
18:3 n3	2.25	0.9	0.93	0.62
18:3 n6	4.50	--	0.11	0.00
20:1	--	0.1	0.10	1.10
20:2 n6	--	--	0.00	0.61
20:3 n6	--	--	0.00	0.42
20:4 n6	24.75	--	0.60	0.59
20:5 n3	--	--	0.00	0.03
22:1	--	--	0.00	0.10
22:4 n6	--	--	0.00	0.21
22:5 n6	--	--	0.00	0.22
22:6 n3	8.98	--	0.22	0.19

Example 5. Preparation of Oil Mix #2 and addition to infant formula.

This mixture represents a totally vegetarian source of long chain PUFAs and would be considered acceptable to persons restricted to a vegetarian diet. This blend is prepared by mixing three parts DHASCO containing about 35% DHA (obtained from *Crypthecodinium cohnii* as described in ~~patent application #07/479,135~~ Example 3) with ten parts ARASCO containing about 33% ARA (obtained from *Pythium insidiosum* as described in ~~patent application~~ Example 2 filed concurrently herewith) and five parts EPASCO containing about 5% EPA (obtained from *N. alba* on deposit with the ATCC as described in ~~patent application 07/496,572~~ Example 1). The resulting mixture, or blend, has the fatty acid composition shown in Table 3. The blend is mixed in a ratio of one part blend to thirty parts of the oils regularly in infant formula. At a normal fat content of 30g fat per liter of Similac® infant formula, this would correspond to the addition of one gram per liter of prepared formula. This supplement provides ARA, DHA and EPA levels equivalent to human breast milk.

Table 3. Composition of a blend of DHA oil, ARA oil and EPA oil in proportions of 3:10:5 by weight.

Fatty Acid	oil mix #2	Infant formula	formula + mix #2	breast milk
8:0 + 10:0	0.00	41.8	40.45	1.74
12:0 + 14:0	16.64	20.7	20.57	14.95
16:0	21.61	6.8	7.28	19.82
16:1	6.55	0.2	0.40	3.20
18:0	0.28	2.3	2.23	5.91
18:1	12.91	10.0	10.09	34.82
18:2 n6	5.87	17.4	17.03	16.00
18:3 n3	1.88	0.9	0.93	0.62
18:3 n6	3.48	--	0.11	0.00
20:1	--	0.1	0.10	1.10
20:2 n6	--	--	0.00	0.61
20:3 n6	0.19	--	0.01	0.42
20:4 n6	18.52	--	0.60	0.59
20:5 n3	0.76	--	0.02	0.03
22:1	--	--	0.00	0.10
22:4 n6	0.11	--	0.00	0.21
22:5 n6	--	--	0.00	0.22
22:6 n3	6.24	--	0.20	0.19

Example 6. Preparation of Oil Mix #3 and addition to infant formula.

This mixture is a blend of ARASCO with fish oils. Oil mixture #3 is prepared by adding one part specially processed Menhaden Oil (Zapata Hayne Inc.) containing about 9% DHA to one part of ARASCO, obtained from *Pythium insidiosum* as described previously containing about 33% ARA. The resultant fatty acid composition is shown in Table 4. This blend is mixed in a ratio of one part blend to thirty parts of the oils regularly in infant formula. At a normal fat content of 30 g fat per liter of infant formula, this corresponds to the addition of 1 gram per liter of prepared formula. This supplement provides ARA and DHA levels equivalent to human breast milk, but the EPA levels are about eight-fold higher than those in breast milk.

Table 4. Composition of a blend of SPMO* and ARA oil in proportions of 1:1 by weight.

Fatty Acid	oil mix #3	Infant formula	formula + mix #3	breast milk
8:0 + 10:0	0.0	41.8	40.45	1.74
12:0 + 14:0	10.2	20.7	20.36	14.95
16:0	15.5	6.8	7.08	19.80
16:1	11.5	0.2	0.56	3.20
18:0	1.41	2.3	2.27	5.91
18:1	8.79	10.0	9.96	34.82
18:2 n6	5.57	17.4	17.02	16.00
18:3 n3	2.31	0.9	0.95	0.62
18:3 n6	3.00	--	0.10	0.00
20:1	0.78	0.1	0.12	1.10
20:2 n6	0.00	--	0.00	0.61
20:3 n6	0.00	--	0.00	0.42
20:4 n6	17.52	--	0.57	0.59
20:5 n3	7.76	--	0.25	0.03
22:1	0.00	--	0.00	0.10
22:4 n6	0.00	--	0.00	0.21
22:5 n6	1.21	--	0.04	0.22
22:6 n3	4.57	--	0.15	0.19

*** Specially Processed Menhaden Oil.**

Example 4. Preparation of Oil Mix #4 and addition to infant formula

Oil mixture #4 was developed to utilize GLA in place of arachidonic acid. This blend was prepared by mixing one part specially prepared Menhaden oil containing about 9% DHA (Zapata Hayne Inc.) with four parts black currant seed oil containing about 18% GLA and one part DHASCO containing about 35% DHA. The resultant fatty acid composition is shown in Table 5. This blend is mixed in a ratio of one part blend to forty parts of the oils regularly in infant formula. At a normal fat content of 30g fat per liter, this would correspond to the addition of 750 mg per liter of prepared formula. This supplement provides EPA and DHA levels equivalent to human breast milk. The ARA levels are about one tenth the level in human breast milk. However, the GLA levels are twenty to fifty times higher than the GLA levels in breast milk which typically are minute.

Table 5. Composition of a blend of SPMO, BCO and DHA oil in proportions of 1:4:1 by weight.

Fatty Acid	oil mix #4	Infant formula	formula + mix #4	breast milk
8:0 + 10:0	0.0	41.8	40.78	1.74
12:0 + 14:0	4.83	20.7	20.31	14.95
16:0	11.86	6.8	6.92	19.80
16:1	2.09	0.2	0.25	3.20
18:0	1.34	2.3	2.28	5.91
18:1	10.98	10.0	10.02	34.82
18:2 n6	31.39	17.4	17.74	16.00
18:3 n3	8.94	0.9	1.10	0.62
18:3 n6	10.60	--	0.26	0.00
20:1	0.26	0.1	0.10	1.10
20:2 n6	--	--	0.00	0.61
20:3 n6	--	--	0.00	0.42
20:4 n6	0.34	--	0.06	0.59
20:5 n3	2.59	--	0.00	0.03
22:1	--	--	0.00	0.10
22:4 n6	--	--	0.00	0.21
22:5 n6	0.40	--	0.01	0.22
22:6 n3	7.51	--	0.18	0.19

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Example 8. Preparation of Oil Mix #5 and addition to infant formula

Oil mixture #5 was developed to best approximate the composition of DHA, ARA and EPA of human breast milk. This oil blend was prepared by mixing one part specially prepared Menhaden oil containing about 9% DHA (Zapata Hayne Inc.) with ten parts of ARASCO containing about 33% ARA and three parts DHASCO containing about 35% DHA. The resultant fatty acid composition is shown in Table 6. This blend is mixed in a ratio of one part blend to forty parts of the oils regularly in infant formula. At a normal fat content of 30g fat per liter of infant formula, this corresponds to the addition of 750 mg per liter of prepared formula. This supplement provides EPA, DHA and ARA levels substantially equivalent to those levels in human breast milk.

Table 6. Composition of a blend of SPMO, ARA oil and DHA oil in proportions of 1:10:3 by weight.

Fatty Acid	oil mix #5	Infant formula	formula + mix #5	breast milk
8:0 + 10:0	0.00	41.8	40.78	1.74
12:0 + 14:0	13.14	20.7	20.52	14.95
16:0	16.83	6.8	7.04	19.80
16:1	8.39	0.2	0.40	3.20
18:0	0.20	2.3	2.25	5.91
18:1	7.66	10.0	9.94	34.82
18:2 n6	6.97	17.4	17.15	16.00
18:3 n3	2.26	0.9	0.93	0.62
18:3 n6	0.25	--	0.01	0.00
20:1	0.11	0.1	0.10	1.10
20:2 n6	--	--	0.00	0.61
20:3 n6	--	--	0.00	0.42
20:4 n6	23.72	--	0.58	0.59
20:5 n3	1.11	--	0.03	0.03
22:1	--	--	0.00	0.10
22:4 n6	--	--	0.00	0.21
22:5 n6	0.17	--	0.00	0.22
22:6 n3	8.35	--	0.20	0.19